## Mineralogical complexity and heterogeneity as a good predictor for biomass colonization in rocks

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Microbes mediate many biogeochemical cycles and their interaction with minerals is essential for understanding nutrients cycling, energy availability and ecosystem dynamics [1]. Microbes can derive energy from minerals, especially in harsh conditions or where conventional energy sources are scarce, leaving a fingerprint of their interactions with the rocks and the environment. Investigating these fingerprints can help assess habitability in extreme environments on Earth while providing insights on analogous conditions that might exist on other planets [2]. However, how the spatial arrangement of distinct minerals within a rock can serve as reliable predictor of biomass accumulation has been poorly investigate so far. We tested the hypothesis that rocks with greater 'mineralogical complexity' may be particularly appealing sites for microbial habitation.

We developed a framework for studying the spatial heterogeneity of distinct minerals measured on the microscale while identifying putative cells. Correlative imaging of mineralogy (via Raman micro-spectroscopy), elemental abundances (via Energy Dispersive X-Ray Spectroscopy, or EDX), and microbial biomass (via confocal laser-scanning fluorescence microscopy) was conducted on three mineralogically distinct substrates: 1) polymetallic nodules from Clarion-Clipperton Zone, 2) carbonate rocks from Point Dume methane seep and, 3) approximately 2 Ky-erupted Iceland basalt.

Section of resin-embedded rocks stained with SYBR green (for overall DNA detection) were imaged and then used to generate Raman maps of minerals, biomolecules, and elemental abundances at microscale. These data sets allowed us to compute "mineralogical complexity" scores across the rocks' surface and verify if biomass, as determined by both organic Raman peaks and SYBR stains, correlated with areas of increased mineralogical heterogeneity.

Our results showed that Raman analysis was able to identify biological fingerprints as confirmed by confocal microscopy. Interestingly, the biomass peaks co-localize with specific mineral-related peaks, suggesting that the spatial distribution of distinct minerals may be predictive of microbial colonization patterns. Our observations indicate that studying microbemineral interactions at microscale can provide insights on microbially mediated transformations and habitability of 'mineralogically complex' samples on Earth and possibly on analog extraterrestrial sites.

Dong et al. (2022), *National Science Review 9*, *N.10*, 1-21.
Chan et al. (2019), *Astrobiology* 19, N.9, 1075-1102.